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THE EFFECT OF DIET OF THE COW AND THE PASTEURIZATION  
OF MILK IN CLOSED BOTTLES UPON THE NUTRITIVE  
AND ANTISCORBUTIC PROPERTIES OF  
COWS' MILK

By

George C. Biggar, B.S. (1921)

A THESIS SUBMITTED TO THE FACULTY OF THE SOUTH DAKOTA  
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PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF MASTER OF  
SCIENCE IN DAIRY  
HUSBANDRY

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## INTRODUCTION

Probably no discovery in the scientific world, and especially in the field of nutrition, has excited more interest in the past few years than the finding and naming of the three hitherto unknown food factors known as vitamins. For many years it has been observed that certain diets for man that apparently are satisfactory as far as their nutritive value is concerned, still seem to lack something necessary for normal growth and function, or else lead to some serious disease. For example it has been known for centuries that sailors on long ocean voyages where the diet consisted largely of dried vegetables and salted meats, were subject to the disease known as scurvy. It was discovered that the addition of lemon juice to the ration prevented and cured this disease. Green foods were found to have a similar curative effect. However little or nothing was done to discover just what the physiological effect of these substances was.

The investigation of the oriental disease known as beri-beri brought about the research work leading up to the present status of vitamins perhaps more than any other one thing. For many years this disease had been prevalent among oriental peoples and its cause was unknown. Investigators finally came to the conclusion that the rice which was a staple article of diet among these peoples had some relation

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to this disease. In the Philippine Islands in 1898 after the United States Government assumed control, one of the first reforms put into operation was the cleaning up of the unsanitary prison conditions. The scientists who were put in charge of this work improved the prison conditions greatly but did not change the prisoners' diets appreciably. The diet consisted largely of polished rice and fish. A great increase of beri-beri occurred which it seemed impossible to check. The investigations following this from 1900 to 1911 finally attributed the disease to a certain substance (later known as vitamine B) which was absent in polished rice but present in rice hulls. In 1906, F. Gowland Hopkins in England, and Eijkman, a Dutch chemist, working along the same lines with fowls came to the conclusion that some unknown food factor was concerned, the absence of which caused beri-beri.

In 1911 Casimir Funk, a German student who had been doing work in producing beri-beri in pigeons, named these unknown dietary factors vitamines. He based the name upon the fact that he believed that nitrogen being present in a basic form made them amines, and being essential to life, he named them Life-amines or vitamines. Hopkins of England named the substances "accessory food factors" but Funk's nomenclature is now generally accepted among investigators. Later investigations brought out the fact that there were three distinct vitamines and these were designated by Dr. E.V. McCollum of Johns Hopkins University as

"fat-soluble A, water-soluble B, and water-soluble C." These names are now used altho Funk has further distinguished them to designate their particular avitaminoses as the antirachitic vitamine (A), antineuritic vitamine (B), and antiscorbutic vitamine (C).

### THE VITAMINES

Fat Soluble A.— This vitamine occurs most plentifully in cod-liver oil and is abundant in other fish oils. Butterfat is a very important source of it as well as egg yolk fat. It is also found in green leaves such as spinach to quite an extent. Domestic animals find an abundance of the substance in clover, alfalfa, and timothy hay, and in yellow corn. This vitamine is necessary to normal growth and its absence often causes the development of an eye disease known as xer<sup>o</sup>thalmia or keratomalacia which may result in blindness. Some authorities assert that rickets is also caused by lack of A. This is probably the case, altho it has been noted that a deficiency of calcium in the diet accompanies lack of A to produce rickets.

Chemically, this factor is quite stable to heat, and ordinary cooking does not cause an appreciable destruction. Oxidation seems to cause a great loss however. Acids and alkalis do not appear to have much effect on this vitamine. It also resists saponification and can be secured in rich portions by freeing fat in this manner. Steenboch at Wisconsin called attention to the fact that fat-soluble A always seems to accompany carotin, the yellow pigment of some



foods such as butter, egg yolk, carrots, and yellow corn, while it is deficient in vegetables lacking this color as in white corn. However this coincidence has thrown little light upon the chemical composition of A.

Most of the experimental work with this vitamine has been done with rats in determining the effect on growth and general condition brought about by the absence of this food factor.

Water-Soluble B.- This vitamine, often called the antineuritic vitamine, is found in many foods, especially plant tissues, all common vegetables and fruits containing the substance. Yeast is the richest source. The germs of cereals also contain much of it.

The absence of water-soluble B from the diet causes principally the disease known as polyneuritis or beri-beri, a disease which results in an atrophy of several organs of the body, mainly the testicles, spleen, ovary, pancreas, heart, liver, kidneys, stomach, thyroid, and brain. It may result in sterility in males and females. While lack of water-soluble B may not cause beri-beri, it may lower the bodily resistance so that other infections may attack the body. Some authorities attribute the spread of tuberculosis during the war to this fact.

Chemically, vitamine B is soluble in water and in 95 per cent alcohol. It is fairly resistant to high temperatures but heating in an alkaline medium seems

to result in a partial loss of its potency. Heating in a neutral or acid medium causes little destruction.

Investigators of the B vitamine have largely used pigeons and fowls with which to carry on their experimental work and have worked especially with rice and yeast as sources of the substance.

Antiscorbutic Vitamine C.— This is the most recently discovered member of the family. It may be stated that its existence was first made known when Holst and Frolich of Norway in 1912 published data showing that fresh potatoes, cabbage, dandelion, carrots, raspberry juices, lemon, and sour dock juice contained a substance which would prevent and cure scurvy. However not all investigators believed in this theory at once and we find that as late as 1917 even so eminent an authority as McCollum (1) made the statement that scurvy in the guinea pig is not the result of the deficiency of a specific protective substance, but rather that the cause of the disease is associated with the retention of feces owing to diets of unfavorable physical character and debility of the digestive tract thru stretching and contact with irritating and toxic factors of bacterial origin. Orange juice, he said, merely acted as a laxative to bring about a cure for the disease. However statements that McCollum has made since that time would indicate that he has changed his views.

The antiscorbutic vitamine is present in fresh vegetables and animal tissues and is most abundant in fresh fruits and green vegetables, and to a lesser



extent in roots and tubers. Orange juice is recognised as being one of the best antiscorbutic substances while lemon juice is also rich in the factor. Such fresh vegetables as cabbage, lettuce, onions, and spinach are excellent antiscorbutics. Tomatoes are also a rich source, canned tomato juice being found by Hess to be as potent as orange juice. Most of the common fruits serve as excellent sources of the vitamins, containing it in varying amounts. Milk has been shown to be of moderate value as an antiscorbutic but its value seems to depend upon the diet of the cow and the treatment of the milk before its use. Another peculiar fact about this vitamin is that while some dried kernels such as barley, peas, and beans do not seem to possess any antiscorbutic properties, yet when germinated the sprouts are fairly rich in vitamin C. It has also been noted that while these same grains are rich in water-soluble B, on sprouting they seem to lose their antineuritic properties. This would indicate an interesting connection between these two vitamins which has not yet been explained.

Comparatively little is known concerning the chemical properties of water-soluble C. It is, of course, as its name implies, water soluble. It is also extractable with alcohol. The substance passes thru a parchment membrane or a porcelain filter. It is not affected by adsorption. Treatment with fullers' earth or colloidal iron does not cause any loss in activity. The ultra-violet light also has no influ-



ence on the vitamine. It is the most unstable of all the vitamins being especially sensitive to heat and alkali. Heating at temperatures above 50° C is destructive to most antiscorbutics and the length of time of heating seems to be an extremely important factor. Oxidation seems also to have a most important effect on the activity of the antiscorbutic vitamine. Rossi (2) noted that guinea pigs fed on oats and hay sterilized in open kettles at 126 C died in 20 days, while with the same materials heated in closed kettles and fed, guinea pigs were alive after two months. Later, (1920) Dutcher, Harshaw, and Hall (3) showed that vitamine C is not destroyed in orange juice by heating at pasteurization temperature (63 C) for 30 minutes or by boiling (100 C) for 30 minutes under reflux condensers, but that by adding hydrogen peroxide as an oxidizing agent, some destructive action took place and still more occurred when the same mixture was heated at 63 C and 100 C. They also showed that orange juice is stable up to the boiling temperature in the absence of oxidizing agents.

The antiscorbutic vitamine has also been found to be much more stable in the presence of acids than in a neutral or alkaline medium. Lemon juice, which contains 7 percent of citric acid, can be heated to 110 C for one hour without any noticeable loss of antiscorbutic potency according to Funk (4). Eddy (5) cites experiments by Sherman, LaMer, and Campbell in which they found that boiling tomato juice at its

natural acidity for one hour destroyed practically 50 per cent of its antiscorbutic power and that boiling for four hours destroyed 70 per cent. Then after neutralizing the natural acidity of tomato juice, boiling it an hour, cooling and reacidifying it, the destructive effect was increased to 58 per cent. After adding N/40 alkali to the tomato juice and boiling for an hour, a 65 per cent destruction was obtained. From 90 to 95 per cent destruction was noted when reacidification was omitted and the neutralized and boiled tomato juice was stored in a refrigerator for five days before using. The latter fact indicates that storing has also a destructive effect upon vitamine C as well as heating and oxidation. Dessication of vegetables has also been found to cause a loss of the antiscorbutic factor, altho the moisture content of the drying chamber and the behavior of different vegetable juices are important factors.

#### VITAMINES IN MILK

Milk has been called the perfect food because of the fact that it contains all the food nutrients present in sufficient quantity to sustain human life. Because of this fact, and in the light of the vitamine hypothesis, it is but natural that the vitamine content of milk and the factors affecting its potency should have been the subject of much research since the vitamine theory has become of so great importance. Funk in 1913 suggested the relationship of the composition of milk to certain deficiency diseases in



children. He pointed out that it was evident that the heating of milk caused the more or less complete destruction of certain vitamins, and also that the vitamin content of the milk depends upon the vitamin content of the food of the mother. Most of the investigation of the vitamin content of milk has had to do with these two factors, namely, the effect of diet of the cow, and the effect of heating and other treatment of milk upon its potency in the prevention of certain avitaminoses.

Effect of Diet of the Cow.- In perusing the literature that has taken up the investigation of the vitamin content of milk from this standpoint, it is plainly evident that there has been a lack of uniformity of results up to this date. This is but natural on account of the many factors which enter into milk composition. There is quite a noticeable variation in the composition of milk even when produced under similar conditions, especially in the protein and fat content. Such factors can be listed as breed of cattle, individuality of cow, period of lactation, quantity of food consumed, composition of the food consumed as produced in different localities, and the care and management of the cattle themselves.

Regarding the influence of the diet of the mother upon the vitamin A and B content of the milk, not as much investigation has been done as has been the

case with the antiscorbutic vitamine. In 1916 McCollum, Simmonds, and Pitz (7) fed female rats on a ration deficient in A and B and observed that the ration was not adequate for the growth of young. However they did not take into consideration the fact that perhaps the milk secretion of the mother rats on the deficient ration was not enough to allow the young sufficient milk for normal growth. In 1920, Hess and Unger (8) suggested that a lack of A in milk might be due to the winter diet of the cow. This seems probable in view of the fact that green feed is lacking in winter. The quality of the dried hays fed is an important factor. In 1921, both Hughes, Fitch, and Cave (9) and Kennedy and Dutcher (10) as the result of experimental evidence concluded that both vitamins A and B were decidedly greater in cows upon a vitamine-rich ration.

The evidence as to the effect of summer and winter rations of the cow upon the antiscorbutic potency of the milk, seems to greatly favor the conclusion that the milk produced under winter feeding conditions is deficient in vitamine C. In 1920, Dutcher, Eckles, Dahle, Mead, and Schaefer (11) concluded that 20 cc. of summer milk was superior in nutritive value and in antiscorbutic potency to 60 cc. of winter milk. They also state that milk becomes poorer in vitamine C very slowly but quickly takes up this factor when green feed is added to the ration.



In a similar experiment at Wisconsin in 1920, Hart, Steenboch, and Ellis (12) showed that winter milk was deficient in C. They state that from 15 to 50 cc. of summer milk is needed to prevent scurvy in guinea pigs and that from 50 to 75 cc. of dry feed milk is needed for complete protection against guinea pig scurvy. Hess, Unger, and Supplee (13) have furnished additional proof of the superiority of summer milk over winter milk. They found that guinea pigs lived an average of 56 days on milk from dry fodder and those fed on milk from fresh fodder lived more than 120 days and then showed only mild evidences of scurvy. It is of interest to note that they also found that the milk containing more vitamin C on analysis showed more calcium and phosphorus and 50 per cent more citric acid than the dry-feed-milk. In contradiction to these statements, Hughes, Fitch, and Cave (9) in 1921 reported that the milk of cows fed a vitamin-rich ration and a vitamin-poor ration showed no difference in regard to the antiscorbutic factor.

Effect of Heating and Other Treatment upon the Vitamines in Milk.— Most of the studies relating to the heating of milk have been made with the antiscorbutic vitamin. It is generally agreed by investigators that the heating of milk results in a more or less complete destruction of the vitamin C so as to cause scurvy when the milk is fed as the only source of this factor. However some believe that

the decrease in the nutritive value as the result of heating cannot be attributed entirely to the effect on vitamins. In 1920, Daniels and Loughlin (14) fed rats on pasteurized milk and noted that they ceased growing. Upon addition of soluble calcium salts such as calcium glycerophosphate to the milk, the rats resumed normal growth. They concluded from this that the loss in nutritive value in pasteurizing milk was due to the precipitation of the calcium salts instead of to a loss of potency of vitamins A. and B. However as they did not concern themselves with vitamin C, no conclusions can be drawn from their experiment concerning this substance.

According to Funk (15), Frolich in 1912 did the first experimental work on the effect of heating on vitamin C in milk. He fed guinea pigs with milk heated at 98 C for 10 minutes with the result that scurvy developed, while heating at 70 C for 30 minutes did not give as certain results. In 1914, Hess and Fish (16) described outbreaks of scurvy in an orphan asylum among children who had been fed with milk heated at 165 F for 20 minutes or at 145 F for 30 minutes. This outbreak showed only a sub-acute form of the disease, the children being restless, irritable, and showed discomfort and retardation in growth and development. The addition of small amounts of orange juice cured these symptoms at once, while the substitution of raw milk for the pasteur-



ized product brought about a cure less rapidly. In 1903, Heubner (17) reported that he had noted a great increase in the number of cases of infantile scurvy in Berlin following the introduction of milk pasteurization which had been in common practice in 1901-02. This pasteurized milk had also been reheated in many cases before using, according to the writer.

The temperature and manner of heating have been shown to be the main factors to be considered in determining the amount of the C factor in milk. In 1919 Barnes and Hume (18) scalded milk by heating rapidly to the boiling point over a gas burner and then quickly cooled it and found that it retained about half of its antiscorbutic potency. Hess in 1920 (19) stated his belief that if the milk was heated in such a manner as to allow oxidation, a much greater loss of the antiscorbutic factor is noticed. He made the statement that "milk pasteurized by simple heating had not lost its potency to nearly the same extent as milk which had been commercially pasteurized for the same length of time and to the same degree." In ordinary commercial pasteurization the milk is often run thru a long series of pipes exposing it in a thin surface to the air. In vat-pasteurization the continuous revolution of the heated coils exposes all the milk to the air.

In 1921, Dutcher and Anderson (20) clearly showed that oxidation is the principal factor in the des-

truction of vitamine C. They found that milk which had been heated in a closed bottle or in a medium of carbon dioxide in the absence of air, retains more of its antiscorbutic potency than milk which has been heated for the same length of time at the same temperature in the presence of air. They also showed that milk heated at 175 F for 5 minutes retains more of its antiscorbutic potency than milk pasteurized at 145 F for 30 minutes, demonstrating the importance of the time factor in heating. The use of hydrogen peroxide as a preservative of milk was shown to be very destructive to vitamine C by these investigators on account of its oxidation properties. Their results with milk heated in an acid medium correspond with those of Dutcher, Harshaw, and Hall (3) working with heated orange juice, in that there was much less destruction of the antiscorbutic substance due to the presence of acid.

Experiments to ascertain the antiscorbutic potency of condensed milks and dried milks all point to the time factor in heating as being immensely important as well as oxidation. In 1919, Hart, Steenboch, and Smith (21) found that unsweetened condensed milk, which depends for its preservation upon concentration and heat treatment only and is given a rigorous heating to insure against spoilage, sustains an almost entire loss of vitamine C, while sweetened condensed milk, which is not subjected to as great heat as the unsweetened product, depending for its preservation upon the sugar present as well as by the concentration



of milk solids, was found by Hess (23) in 1921 "to retain the larger part of its antiscorbutic factor", and by Hume (23) in 1921 to contain vitamine C in apparently undiminished amounts.

In the manufacture of dried milk, two main processes are in use, the Just-Hatmaker process, invented in America but used largely in Europe, and the spray process, the principal method used in America. In the Just-Hatmaker process, the milk is first condensed and then placed in the desiccating machine which consists of two large revolving polished rollers or cylinders placed parallel in a frame. The rollers are about sixty inches in length and twenty-eight inches in diameter. They revolve in opposite directions at about six revolutions per minute. Steam is introduced thru the end of the spindles and a pressure of 40 pounds maintained, insuring a constant temperature of 285 F (126 C). The two cylinders are about one eighth of an inch apart. When the milk falls and spreads in thin sheets on these revolving cylinders, it dries almost instantly. The residue remaining on the cylinders is scraped off in a powder-like consistency by scrapers attached to the machine. The powder is then passed thru a small sieve and is ready for packing.

In the spray process the milk is first condensed in a vacuum pan and then forced in a spray form into

the powder room by means of a pump developing a pressure of 2,000 pounds per square inch. A continuous current of hot air of about 149 C temperature is forced into the powder room at the rate of 14,000 cubic feet per minute which immediately falls to 82 C when it comes in contact with the milk spray. A gradual reduction of temperature to 55 C - 65 C follows. While all manufacturers using the spray process do not use the exact procedure outlined above, the essential points are similar in the various factories where this process is employed.

It can be seen that the powdered milk manufactured by the spray process is heated at a higher temperature and for a longer time and is exposed in powdered form to the air much longer than dried milk produced by the Just-Hatmaker process. This would indicate that more oxidation had taken place, which together with a longer time of heating, would point to a greater destruction of the antiscorbutic vitamin. This has been proven to be the case. In 1919 Hess and Unger (24) reported that milk dried according to the Just-Hatmaker process is equally as protective against scurvy as fresh milk. Hart, Steenboch, and Ellis in 1921 (25) in experiments with guinea pigs using both kinds of dried milk, concluded that the antiscorbutic potency of that produced by the Just-Hatmaker process is decidedly greater than the dried milk produced by the spray process.



Summary of Previous Experiments Affecting the Antiscorbutic and Nutritive Properties of Milk.-

1. The amount of the antiscorbutic vitamine in milk seems to depend upon the diet of the cow, summer or green-feed-milk being superior to winter or dry-feed-milk.

2. The heating of milk in air results in a greater or lesser destruction of the antiscorbutic vitamine, the degree of destruction depending upon the temperature and length of the heating period, i.e. milk heated at a higher temperature for a short time retains more of its antiscorbutic potency than milk heated at a lower temperature for a long time.

3. Milk subjected to oxidation by the addition of hydrogen peroxide or by exposure to the air during pasteurization, suffers a much greater loss of antiscorbutic potency than milk protected from oxidation by heating in the absence of air.

4. Heating milk in an acid medium results in a lesser destruction of vitamine C than when in an alkaline or neutral medium.

## SCURVY

History.- Scurvy has been known to have existed in the world for hundred of years. It was described as early as the thirteenth century and has been noted to have made its appearance particularly on long ocean voyages, polar expeditions, and during wars. Captain Cook (26) on his ocean expeditions in 1768-75 mentioned that scurvy was prevented on these voyages by raw sea-lion meat on one occasion, and by a malt infusion on a second voyage. In 1804, a daily portion of lime juice was introduced into the British navy and according to Budd (27) the number of cases of scurvy decreased greatly.

Scurvy has always accompanied wars and has taken its toll of men by death and disability. Records of the British army in India in 1833-34 showed numerous cases of the disease which was cured by a native sour plant. In the Civil War in the United States (28) there were 30,714 cases of scurvy due to the use of dried vegetables in winter. Similar accounts of the disease are found in connection with the Franco-Prussian War of 1870-71 and the Russo-Japanese War of 1904-05. In the late World War practically all of the armies suffered more or less from the disease. Perhaps the disease was most prevalent in 1916 among the Indian troops in Mesopotamia (29) where it is said that from 30 to 50 per cent of them suffered from scurvy. This was due to



lack of fruit and vegetables during that year. The British troops did not have many cases of the disease because of the fact that they had fresh meat which the Indians would not eat because of their religious beliefs. Because of the seriousness of this outbreak, the British government sent a gardeners' corps of 256 men to Mesopotamia to plant vegetables and to distribute seeds to be sprouted when there was need. A remarkable decrease in the number of cases of scurvy followed. Especially serious outbreaks were common among the Russian, French, German, Italian, and Austrian armies, notably in the prison camps where the diet was naturally less satisfactory than in the regular camps. It is of interest to note that comparatively few cases of scurvy were noted among the American troops, due both to the short duration of their foreign war career and to the excellent commissary department of the United States Army.

Among the civilian population of the world, scurvy has been noted to follow a scarcity of potatoes and fresh vegetables. In England in 1917, cases of scurvy appeared in the poorhouses of Glasgow (31) and Newcastle (32) which was attributed to a scarcity of potatoes. In 1915, Hopkins (33) described 3000 cases of scurvy among the 10,000 population of Aruba, a small island in Dutch Guiana. The disease was caused by a total crop failure in the years 1912 to 1914. The natives subsisted on corn, corn meal, salt fish, and a small amount of dried and salted meat.

The well-to-do class which imported vegetables and fruits did not contract the disease. With the production of a good crop and the availability of fresh vegetables, the disease disappeared. Frolich (34) in 1922 in Norway noted that infantile scurvy occurred in children under good hygienic conditions more than among other children. He stated that this was probably due to the fact that they were fed on prepared foods and well-sterilized milks, while the poorer children, eating a greater variety of food and more raw foods, did not get the disease. Such cases as the latter would indicate that too much cooking and sterilization of foods may not always bring the best results and that the raw food faddists have some ground to their beliefs that raw foods are beneficial to the body.

Etiology: Many theories have been advanced as to the cause of scurvy. Among these are the infectious disease theory, which accounts for the disease as a contagion; the toxic theory, which seeks to account for the cause of scurvy by toxins produced by the consumption of damaged foods; the potassium deficiency theory, which asserts that the curative effects of fruits and vegetables are due to their rich potassium supply; the citric acid theory, which is associated with the antiscorbutic properties of the citrous fruits; and the theory which accounts for the disease by a break in the carbohydrate metabolism, causing an interference with the normal functions of calcium. Most of these theories have been disproved and abandoned, and the vitamine



theory is now generally accepted, accounting for scurvy as a deficiency disease.

Symptomatology.— In adults the symptoms of scurvy manifest themselves first by languor and depression, accompanied by a peculiar paleness of skin. The skin becomes yellowish, dry and scaly, and the lower extremities are covered with petechiae and larger livid spots, evidences of subcutaneous hemorrhages. Pains, similar to those in rheumatism, affect the legs; there is a swelling of the joints; Heart weakness and dyspnea occur; there is a rapid pulse; in severe cases there is soreness and hemorrhages of the gums and looseness of the teeth and falling out of the teeth. Emaciation and atrophy of muscles occur in the later stages. Death finally occurs from complications of an infectious nature or from heart failure.

Infantile scorbutic symptoms are similar to those in adults. They seldom manifest themselves before the sixth month. Before this time it may exist in a latent form, when there is little or no gain in weight, the face is pale or sallow with slight edema of the eyelids, and a poor appetite accompanied with irritability. The acute form of the disease is characterized by anemia, pallor, no gain in weight, rapid pulse, exaggerated knee jerks and general irritability. There is a marked swelling and great tenderness of the joints, thighs, and eyelids, and sometimes soreness and a hemorrhagic condition of the gums.

In guinea pigs, acute symptoms of scurvy usually appear from the fifteenth to the twentieth day on a scorbutic diet. The symptoms manifest themselves by tenderness of joints of the wrists, ankles, and knees. In a day or two there will be a gradual swelling of affected joints, and the pig will wince and cry out in pain when the limbs are touched. There is a roughness of fur, hunched position, inactivity and lack of appetite, and of course a gradual loss in weight. Fractures of bones are often noted among younger animals, while the older ones develop a difficulty in using the hind legs which seem to become stiff or paralyzed. To relieve the pain, the animal lies on its back or on its side in the so-called scurvy or "face-ache" position. There is often a loosening or breaking off of teeth and a hemorrhagic condition of gums. Autopsies show hemorrhages around the joints, in the bone marrow, muscles, and under the skin. Other organs may show congestion or ulceration. Another characteristic symptom of scurvy is the swellings or bead formations of the costochondral junctions. Enlargements of the joints on the limbs are often present.

It must not be expected that all the symptoms enumerated above are to be found in all cases of scurvy. Different individuals may have a great variance in the symptoms noted but in general when the condition of the individual corresponds in part to the symptoms given, the case can quite reasonably be diagnosed as scurvy.



## PURPOSES OF EXPERIMENT

The objects of the experiment as carried on were as follows: To determine the nutritive and antiscorbutic value to guinea pigs of different amounts of milk when produced under the following conditions:

1. From cows which had never received green feed as a part of their ration.
2. From cows in the general college herd, handled and fed under winter conditions.
3. From the cows designated in (1) above after pasteurizing the milk in closed bottles at approximately 145 F (49 C) for thirty minutes.
4. From the cows designated in (2) above after pasteurizing the milk in closed bottles at approximately 145 F (49 C) for thirty minutes.

The outline of the purposes of the experiment assumes that there are differences in nutritive and antiscorbutic properties of milk produced by cows which had never received green feed and cows which received green feed in the summer, but which were under winter feeding conditions during the experiment. The purposes further indicate that the pasteurization of the milk from the cows fed as indicated above may produce differences in nutritive and antiscorbutic properties of the milk.

## EXPERIMENTAL

The Plan: The experimental work was carried out in two periods in order to have a check upon the work. For purposes of explanation, the two trials will be referred to as Period I and Period II in the discussion. Period I covered 120 days, extending from December 12, 1921 to April 11, 1922, while Period II extended from March 6, 1922 until May 25, 1922, a total of 80 days.

The Animals: The guinea pigs used probably averaged larger in size than has been the case in similiar experiments but this fact should have little effect upon the results outside of delaying the onslaught of disease and making the symptoms less severe because the older animals have more resistance. In Period I the animals ranged from 366 grams to 793 grams, with an average of 507 grams.

Those in Period II averaged somewhat larger, the average weight being 580 grams, with a range of from 494 to 658 grams. All the animals were in good physical condition at the beginning of the experiment, having been kept under observation for twelve days previous to the beginning of the experiment to note any abnormal growth changes, or other abnormalities which would affect the final results.

The Quarters: The quarters of the experimental animals consisted of a composite cage which was divided into sixteen individual pens, twelve by sixteen inches in size. (See Plate I, Fig. 1.) The back of the cage was entirely of screen as were also the doors to each pen with the exception of the wooden framework. This allowed the animals



plenty of air at all times.

The guinea pigs were arranged in their respective groups according to weight as closely as possible, one heavy, one light, and two medium-sized pigs comprising each group. In Period I there were six males and ten females, while Period II contained ten males and six females. They were arranged according to sex as nearly as practicable each group containing one or more animals of each sex. This arrangement was necessary in order to bring to a minimum the opportunity for weight or sex difference affecting the outcome.

During the first eleven days of Period I the experimental cage was located in the college dairy barn but on account of the great variation in temperature and the dampness of this location, it was moved to the unused chemical laboratory in the college dairy building which provided warm and quiet quarters for the animals. During Period II the animals were quartered in the chemical laboratory during the entire period. These factors are of assistance in accounting for the difference in results obtained in the two periods especially among the pasteurized milk lots, some animals in which died of pneumonia or other complications.

The pens were cleaned out once and sometimes twice each week and thoroughly washed and disinfected with a solution of creosol or carbolic acid weekly. Clean sawdust was used for bedding, because of its efficiency in absorbing moisture.

Basal Ration: The animals were fed upon a basal ration of whole oats and autoclaved alfalfa hay, ad libitum.

Chopped alfalfa hay was fed after it had been autoclaved at 250 F for thirty minutes and dried. The alfalfa hay was of uniform quality thruout the experiment. This ration furnished the animals sufficient roughage and a fairly good nutritive ratio, altho rather high in protein content. Water was furnished the pigs at first but it was found that they did not drink it on account of the milk fed twice daily.

Experimental Feeding: The feeding of the experimental animals may be outlined as follows for the two periods:

Group I-Animals 1-5-9-13-17-21-25-29-----Received  
30 cc. of milk daily.

Group II- Animals 2-6-10-14-18-22-26-30----Received  
45 cc. of milk daily.

Group III- Animals 3-7-11-15-19-23-27-31----Received  
60 cc. of milk daily.

Group IV - Animals 4-8-12-16-20-24-28-32----Received  
75 cc. of milk daily.

The groups were divided into lots, receiving milk from different sources;

Lot I- Animals 1-2-3-4-17-18-19-20-----Received  
milk from cows always on dry feed.

Lot II- Animals 5-6-7-8-21-22-23-24-----Received  
milk from college herd on winter ration.

Lot III-Animals 9-10-11-12-25-26-27-28-----Received  
pasteurized milk from same cows as Lot I.

Lot IV- Animals 12-13-14-15-16-29-30-31-32-Received  
pasteurized milk from same cows as Lot II.

Period I animals are numbered from 1 to 16 and those in Period II from 17 to 32.



The Cows: The milk fed to Lots I and III was obtained from three Holstein grades, Numbers 306, 307, and 308. These cows had always been on a dry-feed ration. They had been on a high and low protein ration experiment until calving, Numbers 306 and 308 having been on a ration high in protein, while Number 307 had been on the low protein ration. The ration during the major part of the experiment, when the milk was fed as indicated above, consisted of oat straw, ground corn, oats, and bran. From February 15 to March 15 this ration was substituted by silage and alfalfa hay, which might be thought to have some effect upon the nutritive and antiscorbutic properties of the milk, altho dried alfalfa hay has never been found to contain vitamine C, and in working with silage in 1921, Ellis, Steenboch, and Smith (35) were unable to find any of the antiscorbutic vitamine and declared that it was destroyed during the fermentation process. It also might be supposed that the milk from these three Holstein grades might show the result of breed differences when the other lots of animals were fed on milk from four breeds. On this point, Dutcher, Eckles, Dahle, Mead, and Schaefer (11) in 1920 were unable to detect any differences in the antiscorbutic properties in milk from a Holstein and a Jersey cow.

Milk from the college herd was used for the experimental feeding of the animals in Lots II and IV. The samples of milk used was received from the production of 13 Holsteins, 6 Jerseys, 2 Ayrshires, and 1 Guernsey. They had been on a winter ration about two months when

period I commenced. This ration consisted of silage, alfalfa hay, bran, ground corn, oats, and oil meal---a typical winter ration for a dairy herd. The roughages, silage and alfalfa hay, were of uniformly good quality thruout the winter. The herd was on the ration mentioned during the entire experiment, altho access was given to green grass during the last ten days of Period II. This was such a short period that the milk should not have been appreciably affected during that time.

The Milk: The milk from the three dry-feed cows was carefully mixed after each milking and a third taken for the daily sample. Half of this sample was fed raw and the other half was pasteurized. An analysis of this milk was made from time to time to determine any change in composition. The most noticeable change was a progressive increase in the protein content during the experiment. The average of four analyses show that the total solids, protein, and fat content are below the average cow's milk. The average composition of the milk from these three cows was as follows:

|                   | <u>Holstein Grades</u> | <u>Average Cow's Milk</u> |
|-------------------|------------------------|---------------------------|
| Total solids----- | 11.94-----             | 12.9                      |
| Protein-----      | 3.12-----              | 3.4                       |
| Fat-----          | 3.3-----               | 3.9                       |
| Sugar-----        | 4.6-----               | 4.75                      |
| Ash-----          | .72-----               | .75                       |

Naturally the milk from the general herd coming from



a greater number of cows would not vary greatly in composition for the winter period and should closely approximate the average composition of milk. The average fat content for the herd milk was about 3.5 per cent for the winter months according to the daily test made by the college creamery which received the milk for distribution. One analysis of protein content showed a percentage of 3.45 per cent which is considerable higher than the average protein content of the dry-feed milk.

The pasteurization of the milk consisted of heating it in closed pint bottles which were placed in the water in the pasteurizing tank and heated with steam at the same time that the rest of the college creamery milk was pasteurized. The pasteurization process consisted in bringing the temperature of the milk up to 145 F with steam, holding thirty minutes and then cooling down with cold water. However it was found that this exact temperature and time was not the same daily. The following table of temperature tests shows the average of ten days' heatings:

Time to heat to 142 F---44-65 minutes;-average 55.

Time to cool to 50 F ---111-171 " ; - " 129

Maximum temperatures recorded-142-146 F- " 144 F

Average time above 142 F-----0--30 minutes.

The above temperatures were taken from milk heated in closed quart bottles. It was found that milk heated in pint bottles (as the experimental milk was heated) averaged 2 F higher than that in the quart bottles.

This would indicate that the experimental milk reached an average of 146 F at each heating and was held above 142 F for thirty minutes. While this variation in heating may not be permissible from a scientific standpoint, still it is the more practical for experimental work as it gives a fair representation of the treatment of milk pasteurized-in-the-bottle as it is carried out at the average milk plant using this method and selling milk for public consumption.

Feeding the Milk: The milk was fed to the experimental animals in aluminum dishes twice daily with regularity, half of the portion being given at each feeding. The feeding hours ranged from 6:45 to 8:00 a.m. and 4:45 to 6:00 p.m. After the milk was consumed, the oats and autoclaved alfalfa were fed, ad libitum. The pigs receiving 30 and 45 cc. of milk per day invariably consumed their portions quickly, except when suffering loss of appetite and sickness. The pigs receiving 60 and 75 cc. portions would not take all of their milk of their own accord and some individuals would take none voluntarily. In such cases it was necessary to force-feed the milk by means of a medicine dropper in order that each pig would get his allotted portion. It was not noted that this method of feeding appreciably affected the growth of the animals. The allotted portions were fed to each pig without fail each day during the entire experiment.

The age of the milk differed in that the raw milk



was one half to one day old when fed while the pasteurized milk was from a day and a half to two days old, the pasteurization process making this necessary. Between feeding periods the milk was kept in cold water or in the ice box to prevent its souring.

Weighing: The animals were weighed every fourth day just before the evening feeding, the scale used being a 2-kilogram Chatillon balance. (See Plate I, Fig.2) The weights of each animal were carefully recorded.

Observation: The experimental animals were under observation most of the time during the day and any unusual condition or occurrence was recorded in the note-book used in recording the experimental data. In this note-book were also recorded all post mortems of pigs. Each pig that died was very carefully examined to determine the cause of death and if death was caused by scurvy, careful note was taken of the symptoms manifested. In the post mortem examination, the organs of digestion were thoroughly examined for any abnormality, as well as the muscles, joints, costochondral junctions, heart, lungs, and mouth. The majority of the post mortem examinations were conducted in the Animal Health Laboratory of the college, and always took place as soon as possible after the death of the animal, usually within four hours.

## DISCUSSION

Group I: Chart I, Group I, Series 1, shows the results of feeding 30 cc. of dry-feed milk both raw and pasteurized. It will be noted that both animals 1 and 9 of Period I gave birth to young on the 16th day. Number 1 gave birth to one little pig weighing 123 grams which was healthy and normal at birth. However the little pig contracted scurvy in two days and died with marked scorbutic symptoms on the fifth day. The presence of an eye infection in this little pig indicated a possible lack of vitamine A in its ration. The mother, Number 1, showed symptoms of scurvy on the 20th day. A restorative diet of orange juice and mangel beets was fed, but her growth fluctuated until the 64th day when she made a distinct upward climb. On the 12th day Number 9 gave birth to two living and one dead pigs. The two living pigs survived only a few hours. The mother showed scorbutic symptoms on the 20th day and she was given the restorative diet on the 24th day. Her growth fluctuated much and she died on the 56th day. Her inability to recover was doubtless due both to the number of young to which she gave birth and to the fact that 30 cc. of dry-feed milk pasteurized did not give much protection from scurvy. This shows a superiority of the raw dry-feed milk the same milk pasteuzized. These results with pregnant mothers correspond with those of Ingier (36) in 1915, who stated that when a scorbutic diet is fed during the latter part of pregnancy, the young were born alive and apparently



fully developed, but with latent scurvy which became acute if the mother continued on a scorbutic diet nursing young. He also observed that pregnant females more quickly succumbed to a scorbutic diet than do animals not subject to the demands of pregnancy or lactation.

In Period II of Group I, Series 1, Number 17 on 30 cc. of raw dry-feed milk shows a remarkably normal growth curve for 80 days with no evidences of scurvy whatever. Upon 30 cc. of this same milk pasteurized, Number 25 showed scorbutic symptoms about the 24th day. She developed the disease very slowly until the 52nd day when she started a distinct downward trend and succumbed to scurvy on the 77th day. Her ability to live such a length of time indicates some antiscorbutic potency in 30 cc. of dry-feed milk pasteurized.

In the corresponding groups fed milk from the general herd, (shown in Chart I, Group I, Series 2) Number 5 on 30 cc. of raw milk gave birth to two young, both of which died within a day. She did not make a normal growth but showed no scorbutic symptoms until the 64th day when she became paralytic and in about 10 days was unable to use her legs at all, lying on her back or side or most of the time. Orange juice revived her in three days and she soon lost all evidence of paralysis. The results with orange juice shows that she had a form of scurvy that is more prevalent among older guinea pigs, that is, paralysis of the limbs with little loss in weight. Number



13 on the pasteurized milk in the corresponding group of Period I manifested scurvy on the 12th day and died on the 25th day with scorbutic symptoms as well as inanition and a peptomized condition of the lungs.

In Period II, Number 21, on 30 cc. of raw herd milk showed a normal growth curve to the 80th day with no scorbutic symptoms. Number 29 on the same milk pasteurized, commenced to lose weight rapidly and showed symptoms of scurvy after the 12th day. His symptoms included inanition as well as scurvy. At the end of the 80th day he was still alive altho having lost 43 per cent of his body weight. Scorbutic symptoms were particularly indicated by his teeth, all incisors being broken off and bleeding of gums occurring. Changing of his ration to 60 cc. of raw milk and green feed showed a great improvement in this pig after the experiment and at this writing he shows evidences of recovery.

A summary of Group I would indicate that 30 cc. of dry feed or general herd milk either raw or pasteurized is not sufficient to protect pregnant guinea pigs from scurvy or allow them to rear young without the latter contracting the disease. It also shows that 30 cc. of dry feed and general herd milk (raw) protects guinea pigs fully for 80 days from scurvy. When this same milk is pasteurized-in-the-bottle, its protective power is diminished and it will not prevent scurvy. A very slight advantage of the general herd milk over the dry feed milk is indicated.

Group II: This group is shown in Chart II. The growth curves show that all animals in Series 1 on the dry feed milk made fairly normal growth except Number 10. This animal showed ema-



ciation after the fourth day and also evidenced symptoms of pneumonia. He died on the 19th day with marked scorbutic symptoms, especially in the limbs. It is believed that his rapid emaciation was not due to scurvy alone. Number 2, fed 45 cc. of dry-feed milk, made a normal growth until the 88th day when she developed paralytic symptoms and soon lost the use of her hind legs and showed swellings of the forelegs. The addition of orange juice and green food caused her partial recovery and her normal growth, but she is still deformed and cannot handle herself well. Her condition is very well shown in Plate I, Fig. 5. The growth curves of animals 18 and 26 show that both raw and pasteurized results with 45 cc. of dry-feed milk check quite closely in Period II.

In Series II of Group II, it will be noted in Period I that Animal 6, while making gains in weight, shows rather peculiar fluctuations. This was due to the fact that his incisors kept breaking off continually. His upper incisors broke off and grew in four times and his lowers showed the same behavior three times. This remarkable happening can doubtless be attributed to a very mild form of scurvy which has been noted to cause brittleness of teeth, altho the tendency for them to grow in again is rather peculiar. Zilva and Wells (37) in 1919 called attention to this decalcification of teeth which they describe as a fibroid degeneration of the pulp in which the

fine cellular connective tissue of normal teeth is replaced by a fibrous structure devoid of distinct cells. In this group, Number 14 died on the 24th day from strangulation during feeding. He showed no scorbutic symptoms. Number 22 of Period II shows a fairly normal growth curve and had no symptoms of scurvy on the 80th day. Animal 30 in Period II, receiving 45 cc. of herd milk pasteurized, evidenced slight scorbutic symptoms about the 12th day. His growth curve fluctuated much and his final weight on the 80th day showed a loss of 8 per cent during the experiment. His symptoms were mainly inactivity and a slightly paralytic condition of the legs.

The results of Group II show a greater protection from scurvy with 45 cc. of milk than with 30 cc. The former amount of both dry-feed milk and herd milk was entirely protective for 80 days in two cases, while symptoms of scurvy appeared in the other two animals. In the pasteurized lots, 45 cc. had a protective effect but was entirely protective only with pig Number 26 for 80 days, doubtless due to individuality. There was a slight advantage in herd milk over dry-feed milk in two animals and an advantage of the dry-feed milk in one instance, that of number 26.

Group III: All of the animals in Group III, Series 1, (shown in Chart III) have quite strikingly normal growth curves, with the exception of Number 11 of Period I, which developed pneumonia and died on the 8th day. No scorbutic symptoms appeared in this series.



In Series 2, both animals in Period II made normal growth curves that coincide remarkably well, showing no difference between the pasteurized and raw milk. These two animals are pictured in Plate II, Fig. 3. In Period I. Number 7 on 60 cc. of raw herd milk made a fairly normal growth curve until the 68th day when she declined rapidly and died on the 76th day. Post mortem examination revealed that the cause of death was fatty degeneration of the liver. Number 15 of the pasteurized lot declined rapidly after the 8th day and death occurred on the 20th day. While she had no external scorbutic symptoms, with the exception of a broken tooth, there were several hemorrhagic conditions shown on post mortem as well as evidences of digestive troubles. The cause of death was probably complications with scurvy. It can be seen that in Group III, 60 cc. of both raw and pasteurized milk was protective against scurvy with the exception of Number 15, where complications doubtless lowered the animal's resistance.

Group IV: In this group which is shown in Chart IV, the results seem to indicate that 75 cc. of dry-feed milk and herd milk, both raw and pasteurized, are protective against scurvy. The curves show a remarkable uniformity except in case of Number 12 on pasteurized dry-feed milk, which died on the 25th day with some scorbutic symptoms. His case was similar to that of Number 15 in Group III in that complications may have caused scorbutic development. Outside of hemorrhagic areas in Number 12, no other symptoms

were manifest. In this group, Number 16 died on the 11th day of pneumonia.

In reviewing the results of the experiment, it must be observed that the lack of uniformity in the two periods was probably due to the fact that the animals in Period I were in rather cold and damp quarters during the first eleven days of the experiment, with the result that some of them contracted the pneumonia and died or else lowered their resistance to such an extent that complications set in. It is also noteworthy that all the animals in Period I that succumbed from pneumonia or scorbutic complications were in the pasteurized milk groups. The results in Period II throw little light as to why this should have been the case.

#### CHECK TRIAL

Owing to the fact that the results obtained in the experiment indicated that 30 cc. of both dry-feed milk and herd milk as produced under winter conditions were protective against guinea pig scurvy and that these results do not check with those in similiar previous experiments such as that of Dutcher, Eckles, Dahle, Mead, and Schaefer (11) who found that 30 cc. of winter milk did not prevent scurvy, it was thought that perhaps the basal diet of oats and autoclaved alfalfa might possess some antiscorbutic potency. Therefore, four animals from the first period of experiment were placed upln the basal ration of oats, autoclaved alfalfa, and water alone. The animals used



were Numbers 3,4,6, and 8, weighing 836, 740, 700, and 660 grams, respectively. These animals had been receiving their experimental allotments of milk for 144 days and had shown no symptoms of scurvy, with the exception of Number 6, who had evidenced very mild symptoms. (Loss of teeth). The uniformity of the growth curves of these four animals may be noted in Chart V.

The outcome of this trial proved conclusively that the basal diet afforded no protection against scurvy. A glance at Chart V will show that there was a rapid decline in weight almost immediately following the changed ration. On the 22nd day Numbers 3,6, and 8 died from severe scorbutic symptoms within six hours of each other. Number 4 was placed upon a restorative diet of orange juice and milk and she survived. The symptoms manifested included roughness of coat, loss in appetite, and swellings on forelegs, and post mortem examination in all cases revealed remarkable brittleness of bone, separation at the joints, hemorrhagic conditions of muscles and other tissues, and beaded costochondral junctions.

Inasmuch as the results found indicate that a basal ration of oats and autoclaved alfalfa is free from the antiscorbutic factor, the only other suggestion that might be made in comparing the results of feeding 30 cc. of winter milk in this experiment and that of Dutcher, Eckles, Dahle, Mead, and Schaefer (11) is that inasmuch as the latter used oats alone as the basal ration, while in this

experiment autoclaved alfalfa was fed in addition to the oats, it is possible that the addition of the alfalfa might result in a more complete metabolism of the food nutrients and would increase the resistance of the animal against scurvy. Such a possibility has been suggested by Pitz (7).

#### SUMMARY AND CONCLUSIONS

1. There is a slight difference in favor of the normal winter ration in comparing the nutritive and antiscorbutic properties of milk produced by cows fed dry feeds and milk produced by cows under usual winter feeding conditions.
2. Protection against scurvy is afforded guinea pigs for at least 80 days by feeding 30 cc. of dry-feed milk or winter herd milk, with a basal ration consisting of oats and alfalfa hay.
3. Pasteurization of milk in closed bottles at approximately 145 F. for thirty minutes results in a partial destruction of the antiscorbutic vitamine, 60 cc. of pasteurized dry-feed milk or winter herd milk being necessary for complete protection against scurvy in guinea pigs, when on basal ration of oats and alfalfa hay.
4. Guinea pigs in the later stages of pregnancy fed 30 cc. of raw or pasteurized milk, fail to have normal parturition, the young either being born dead or contracting scurvy soon after birth.
5. Oats, and alfalfa (autoclaved at 250 F for thirty minutes) which was used as a basal ration, furnished no protection against scurvy in guinea pigs.

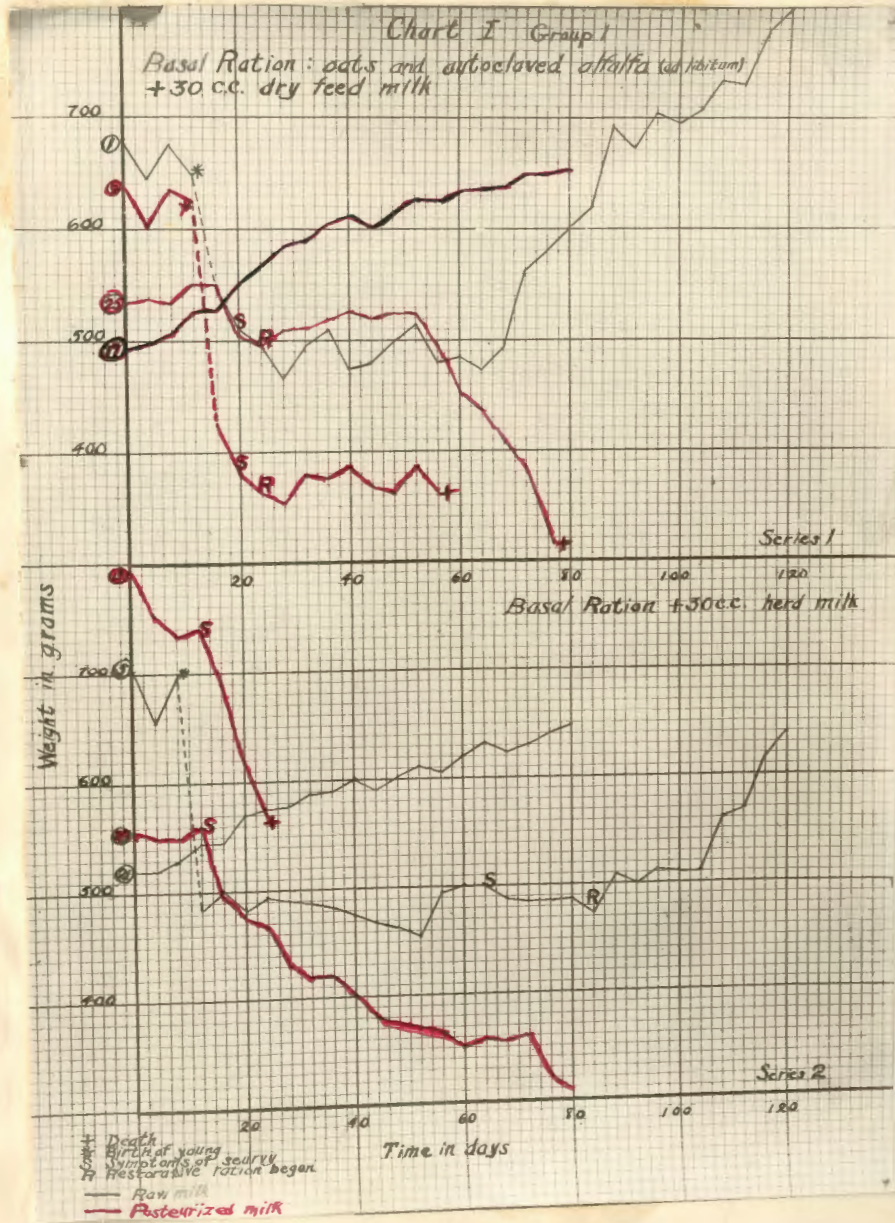


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In conclusion, I wish to acknowledge the assistance of Dr. J. B. Taylor for his aid in some of the post mortem examinations and of Professor T. H. Wright, Jr. for data relative to the pasteurization of milk.

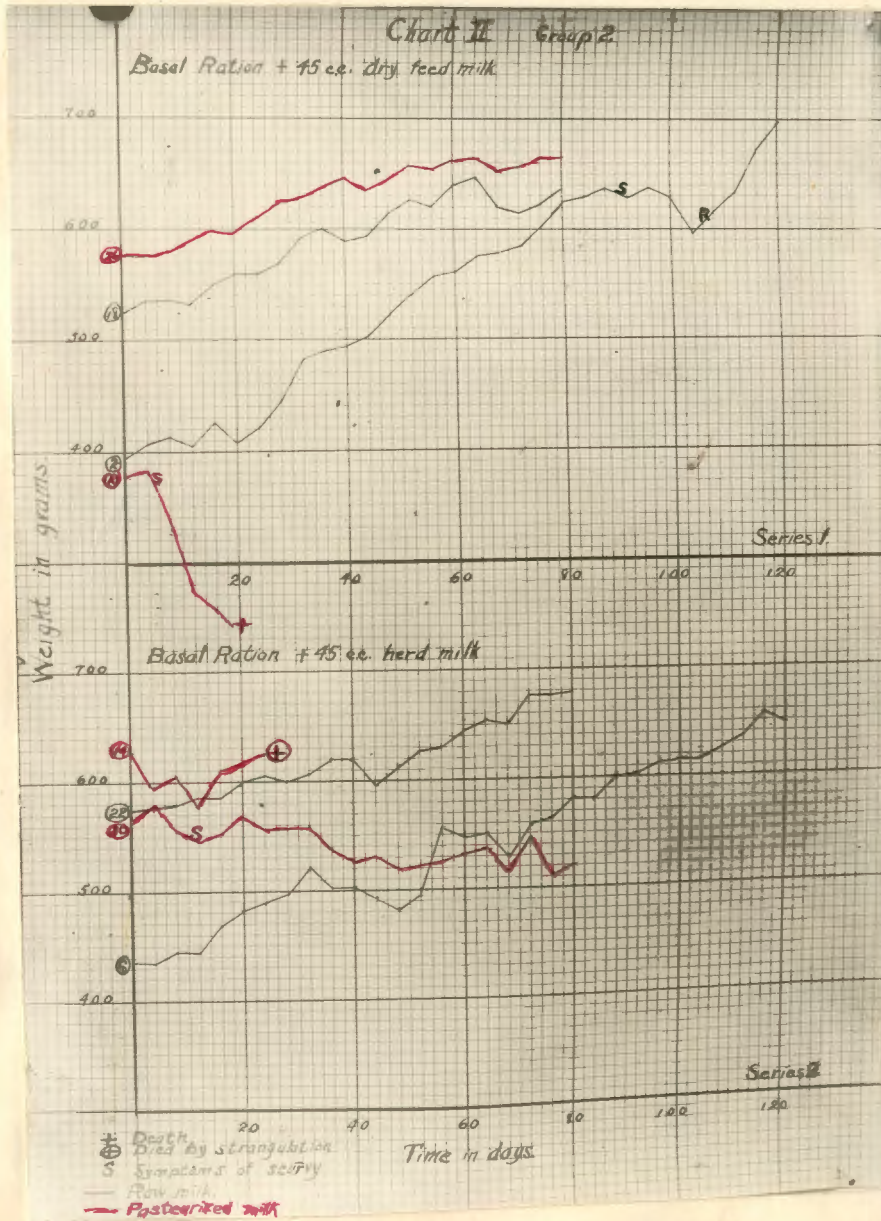
Special acknowledgment is due Dr. Willis E. Johnson who made it possible for me to pursue graduate work, and to Professor Thomas M. Olson, under whose guidance and direction the experimental work was carried out.

# Chart I, Group 1.

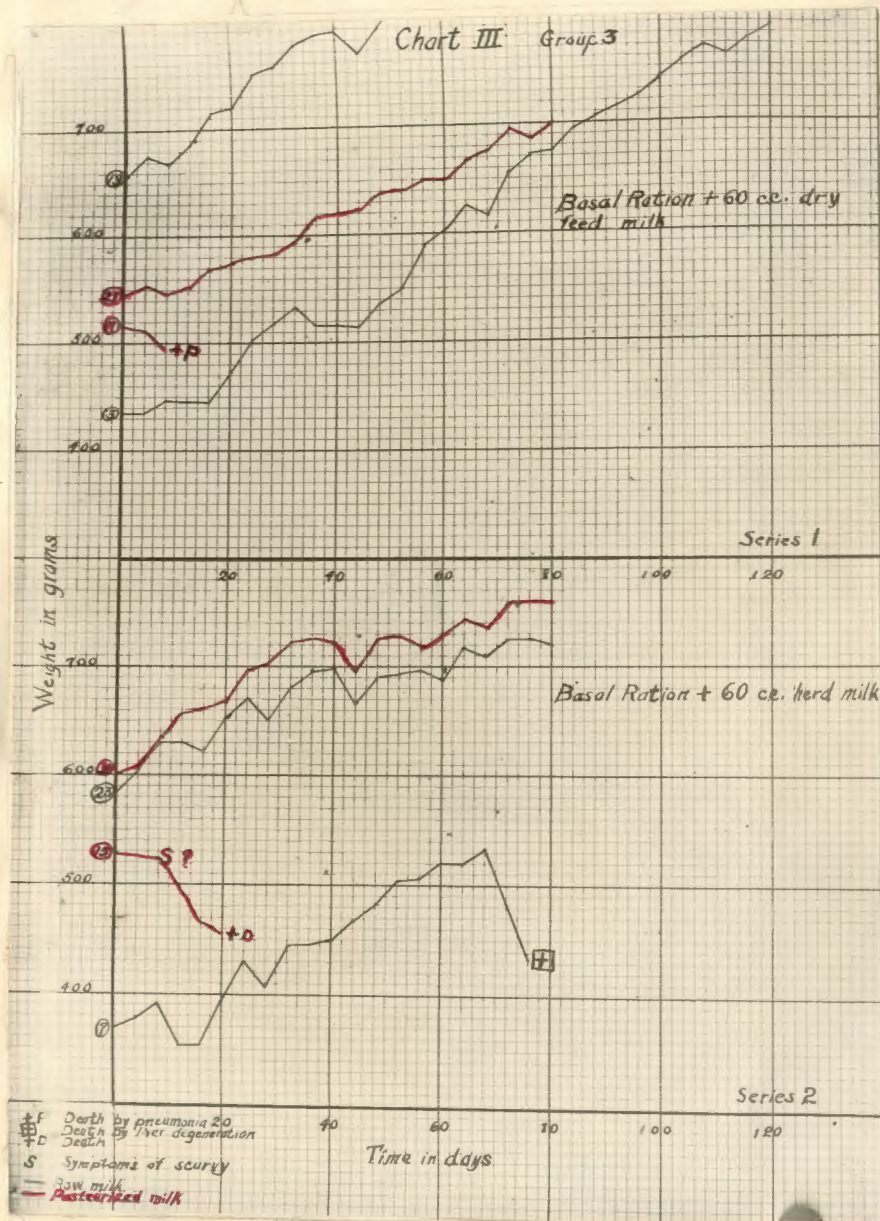




# Chart II, Group 2.

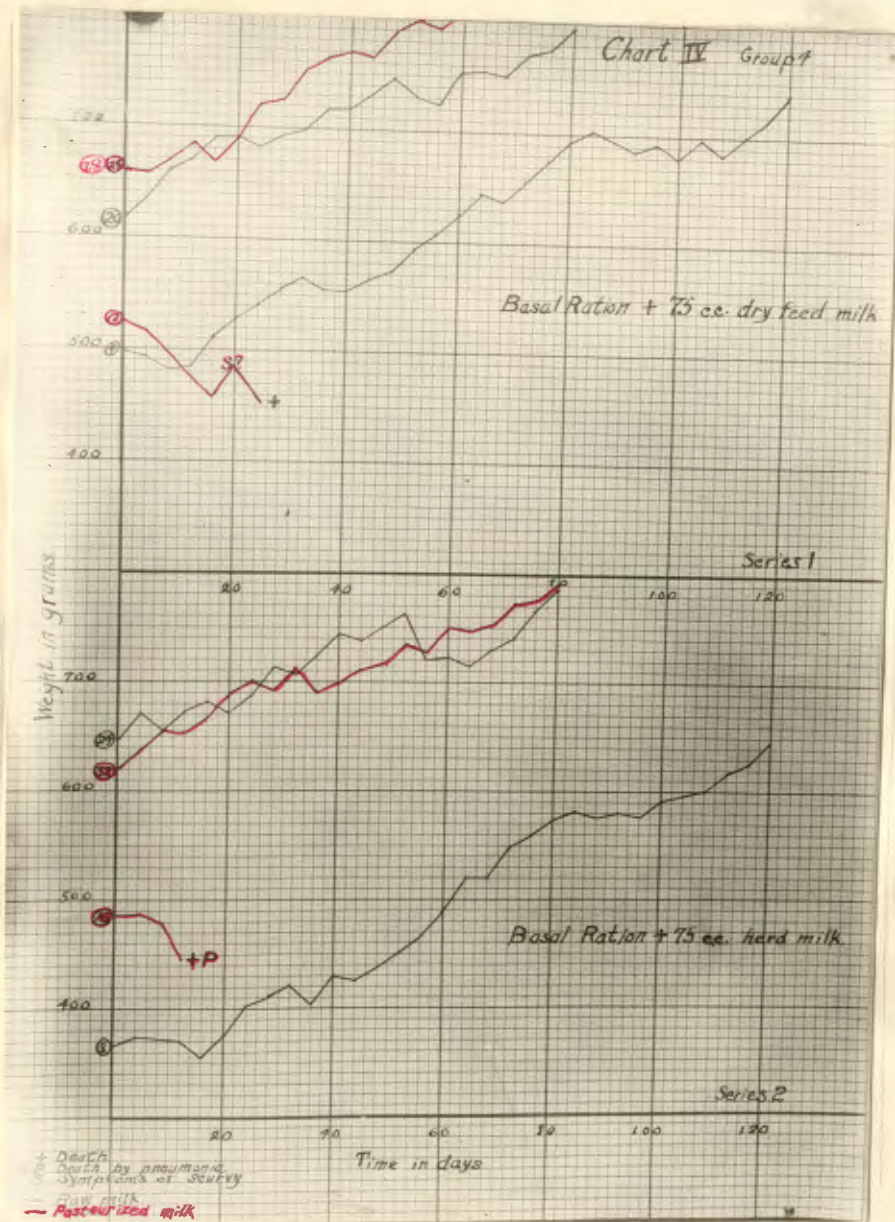


# Chart III, Group 3.





# Chart IV, Group 4.





# Chart V

Showing Result of Changing Diet of Four Guinea Pigs from Basal Ration + milk to Basal Ration Alone.

Basal Ration: Oats + autoclaved alfalfa, ad libitum,

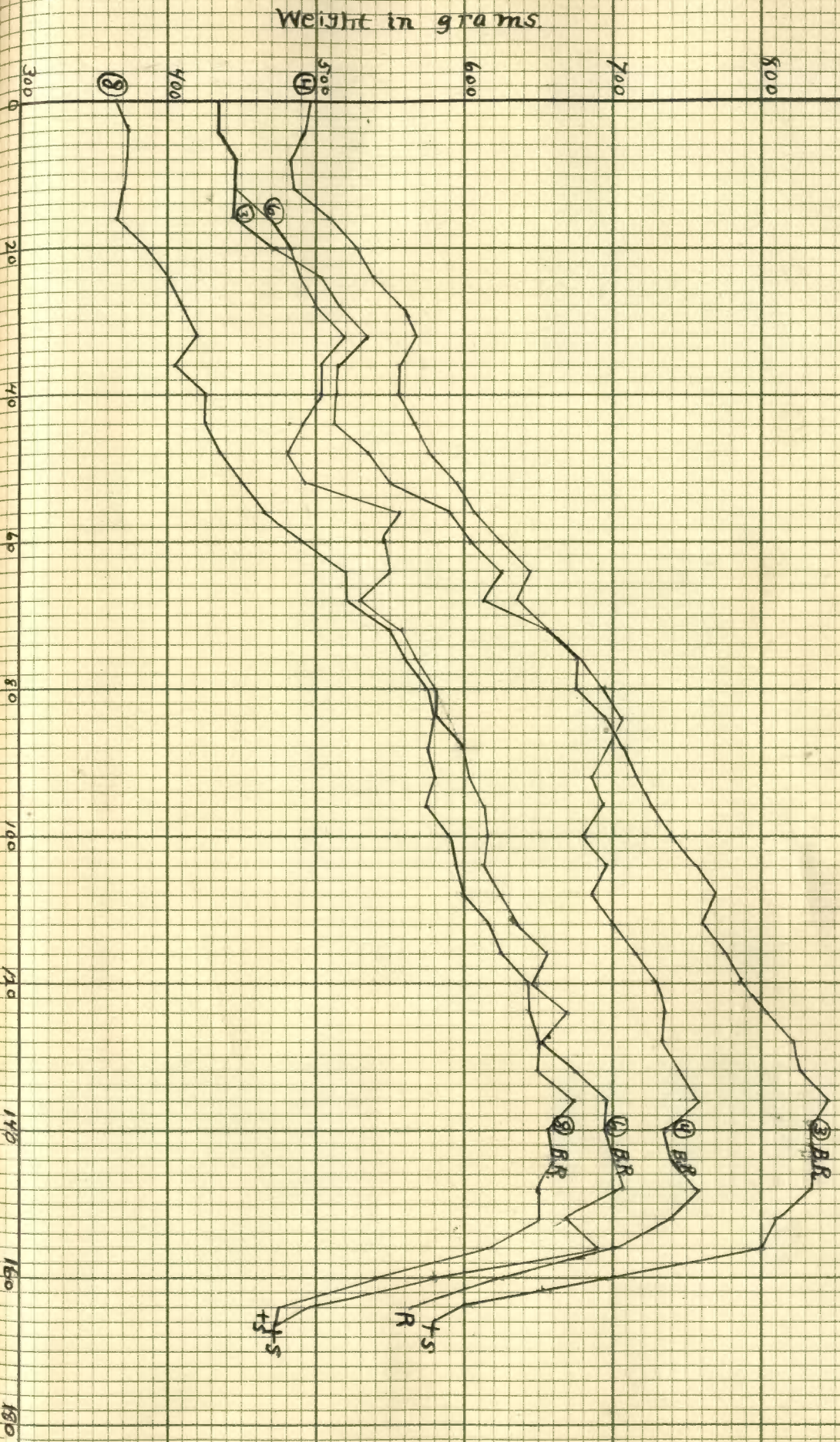




PLATE I



Fig. 1.

Showing cage used for experimental animals. The doors (open) are covered with screen as well as the back of the cage.



Fig. 2.

An illustration showing how the animals were weighed on a 2-kilogram Chatillon balance. Weighings were made every 4th day.



Fig. 3.

A healthy, normal, guinea pig. This is a likeness of Number 31 at the close of Period II.



Fig. 4.

A scorbutic animal showing hunched position, roughness of coat, and emaciation.-No. 29.



Fig. 5.

Number 2 of Period II showing paralytic condition contracted after 80th day. No complete recovery.

PLATE II



Fig. 1.

A comparison of the corresponding animals on 30 cc. of herd milk. 21-raw milk; 29-past. Original weights: 21-520 gms.; 29-553 gms. Final weights, (80 d's): 21-647 gms. 29-310 gms. Animal 21;-normal growth; no scorbutic symptoms. Animal 29;-scurvy and inanition.



Fig. 2.

A comparison of the corresponding animals on 45 cc. of herd milk. 22- raw milk; 30-past. Original weights: 22-574 gms.; 30-566 gms. Final weights;(80 d's): 22;677 gms.;30-520 gms. Animal 22;-normal growth, no scorbutic symptoms. Animal 30:- fluctuating growth. scorbutic symptoms.



FIG. 3.

A comparison of the corresponding animals on 60 cc. of herd milk. 23-raw milk; 31- past. Original weights, 23-587 gms.; 31-600 gms. Final weights:(80 d's), 23-760 gms. 31-720 gms. Animal 23;normal growth; no scorbutic symptoms. Animal 31; normal growth; no scorbutic symptoms.



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